

Whole sediment Toxicity Identification And Evaluation (TIE) Assessments in Baltimore Harbor

Presented by:

**Dan Fisher
University of Maryland
Wye Research and Education Center**

Why Whole Sediment TIEs?

Initial TIE assessments focused on interstitial waters

Methods were a fairly straightforward application of established effluent TIE methods to the interstitial water phase of sediments

Because of the process necessary to isolate interstitial water from bulk sediments, there are many issues that complicate interpretation of interstitial TIEs

- **Changes of metal toxicity due to oxidation during routine interstitial toxicity tests**
- **Changes in interstitial pH due to CO₂ volatilization**
- **Underexposure to high log K_{ow} compounds that may sorb to glass test containers**
- **Overexposure of animals not normally exposed to 100% interstitial water**
- **Elimination of other routes of exposure such as sediment ingestion**
- **Ammonia toxicity may be overemphasized because these tests generally overexpose organisms to water soluble extracts**

Why? Continued

Due to these problems, scientists at the Atlantic Ecology Division of the US EPA (Narragansett, RI) who were instrumental in the original development of the marine pore water TIE procedures began development of marine whole sediment TIE procedures.

The methods have now been finalized and have been published or are currently submitted for publications. They have also been validated using both environmental samples and spiked sediment exposures.

These are the methods I will present in the following summary. They involve three sediment manipulations:

- Powdered coconut charcoal (PCC) is added to one treatment to remove organic compounds**
- Chelating resin is added to one treatment to remove metals**
- Zeolite is added to one treatment to remove ammonia**

Initial Experiments

The US EPA methods were originally designed using the estuarine amphipod *Ampelisca abdita*. Tube dwelling amphipod tested at 20°C and 20 to 30‰. Not resident in Chesapeake Bay. Field collected, not cultured in the laboratory.

Since all of the Baltimore Harbor sediment toxicity data is based on the indigenous tube dwelling estuarine amphipod *Leptocheirus plumulosus* we propose to conduct the TIE studies with this organism at 25°C and 5‰.

Initial set of experiments designed to test whether *L. plumulosus* is suitable to testing using the small test beaker size (100 mL), sediment volume (20 g), shorter exposure period (4 d vs 10 D) and sediment manipulations used in the US EPA methods with *Ampelisca abdita*.

Will conduct a series of tests using sediment from three areas in Baltimore Harbor of known contamination and toxicity.

Initial Experiments (Cont.)

Experiments:

Compare survival in control sediment in small beakers to that in normal 1 L beakers. Crowding should not be an issue since these amphipods are found in dense populations in the field. In addition, the amphipod density in the 100 mL beakers with 20 g of sediment ($0.51/\text{cm}^2$) is well below a published density that results in adequate survival and reproduction ($1.4/\text{cm}^2$) in a laboratory test.

Compare whether toxicity of natural contaminated sediments is similar between a 10 d test using large beakers and a 4 d test using the small beakers. Sediments from Baltimore Harbor with mostly organic contamination, mostly metals contamination and a mixture of contaminants will be tested. Toxicity was the same when these types of comparative tests were conducted with *Ampelisca abdita*.

Compare whether survival of *L. plumulosus* in the small beakers with uncontaminated sediments is adequate when the sediment manipulations are conducted.

Initial Experiments (Cont.)

Determine whether the sediment manipulations work for *L. plumulosus* in natural sediments with known contamination and toxicity. Sediments from Baltimore Harbor with mostly organic contamination, mostly metals contamination and a mixture of contaminants will be tested.

All of these tests will be conducted at 25°C and 5‰. Tests will be static with aeration but no feeding. Three replicates will be used in all treatments. There will be 20 amphipods per replicate in the 1 L test beakers and 10 amphipods per replicate in the 100 mL beakers.

TIE Tests - General Conditions

All test for the various manipulations for each sediment will be conducted at the same time

Test type:	Whole sediment, static
Temperature:	25 ± 1°C
Overlying water:	5 ‰
Light Quality:	Ambient laboratory light
Photoperiod:	16:8 (L/D)
Test chamber:	100 mL beaker
Sediment amount:	20 g
Overlying water volume:	60 mL
Species:	<i>Leptocheirus plumulosus</i> (1 - 7 d old)
# replicates:	3
# amphipods/rep:	10
Feeding:	Not fed
Aeration:	Gentle (1-2 bubbles/sec with pipette)
Test duration:	4 d
Endpoints:	Survival
Performance criteria	Control survival ≥ 80%

TIE Manipulations – Coconut Charcoal (PCC)

PCC will be purchased from Calgon Corp., Pittsburgh, PA.

PCC hydrated before using by combining 290 grams dry PCC with 1000 mL deionized (DI) water

Excess water removed by centrifugation (4,000 rpm for 30 min. at 4°C)

Forms a black paste that can be stored for up to 6 months in a tightly covered container at 4°C in the dark

Prior to test, 3.0 g of wet PCC paste is added to every 20 g of the wet sediment to be tested and is mixed thoroughly with a stainless steel spoon

20 g of PCC/sediment mixture added to each beaker and clean overlying water is carefully added to minimize sediment resuspension

Beakers are then gently aerated for a 24 h equilibrium period before organisms are added

TIE Manipulations – Metals Chelating Resin

Resin Tech™SIR-300 (high purity) will be purchased from Resin Tech, Inc., Cherry Hill, NJ

The resin is rinsed five times with DI water, then five times with test water, and then stored in test water at a ratio of ~one part resin to three parts water at 4°C in the dark

Immediately before use (~24 h), three more rinses about 1 h apart with test water at the 1:3 ratio will be done

Prior to test, 5.0 g of resin is added to every 20 g of the wet sediment to be tested and is mixed thoroughly with a Teflon coated spatula

20 g of resin/sediment mixture added to each beaker and clean overlying water is carefully added to minimize sediment resuspension

Beakers are then gently aerated for a 24 h equilibrium period before organisms are added

TIE Manipulations – Metals Chelating Resin

Zeolite (SIR-600) will be purchased from Resin Tech, Inc., Cherry Hill, NJ

Material is used without further preparation

Prior to test, Zeolite is added directly to the sediment at a proportion of 20% (by wet weight) and is mixed thoroughly

20 g of Zeolite/sediment mixture added to each beaker and clean overlying water is carefully added to minimize sediment resuspension

Beakers are then gently aerated for a 24 h equilibrium period before organisms are added

TIE Tests – Pore water

A pore water toxicity test will be conducted on each of the whole sediments. This Information is necessary because if further TIE phases are needed later they can currently be done only on pore water.

Test type:	Pore water, static
Pore water collection:	Centrifuge (5,000 g for 2 h)
Temperature:	25 ± 1°C
Overlying water:	5 ‰
Light Quality:	Ambient laboratory light
Photoperiod:	16:8 (L/D)
Test chamber:	25 mL beaker
Test volume:	20 mL
Species:	<i>Leptocheirus plumulosus</i> (1 - 7 d old)
# replicates:	3
# amphipods/rep:	5
Feeding:	Not fed
Aeration:	None
Test duration:	48 h
Endpoints:	Survival
Performance criteria	Control survival ≥ 90%

Bioaccumulation Tests

One of the problems with the short-term TIE procedure is that it is not designed to give information on the bioaccumulation of contaminants by sediment dwelling organisms.

Published information (Lee et al., 2000) indicates that some benthic organisms are able to accumulate metals from whole sediment through ingestion, regardless of the AVS-SEM values. Excess sulfides do not protect against bioaccumulation from sediment ingestion.

Seems to be somewhat metal/organism specific. Ni, Cd, and zinc accumulation correlated with total extractable metals not pore water for deposit and filter feeding clams but Cd accumulation governed by pore water for a surface deposit feeding worm

Deep deposit feeding worm did not accumulate Cd but did accumulate Ni and Zn in proportion to total extractable metals and pore water AVS.

Apparent that there can be bioaccumulation in sediments with high sulfides. Lee et al. feel this may be more important for lower level exposures and chronic effects.

Bioaccumulation Tests (Cont.)

At a subset of three TIE sites we will determine the bioaccumulative potential of the sediment contaminants by *L. plumulosus* which is a suspension surface and deposit feeder and the polychaete *Nereis virens* which is a sediment deep deposit feeder.

Tests will be conducted on sediments that have not caused a dramatic survival effect in past tests but which have elevated levels of bioaccumulative contaminants. Sites will be picked that have predominately different types of contaminants.

Examples of possible stations:

BSM 7 has Ni, Pb, and Zn levels above the ERM values

BSM 19 was not acutely toxic but had some PAHs above ERM values, as well as Ni and Zn

BSM 65 has PCBs, Ni, and Zn above the ERM values

If you notice a pattern here it is fairly obvious that there are a lot of stations with metals concentrations above ERM values. It is difficult to find a station with low metals concentrations.

Bioaccumulation Tests (Cont.)

Tests will generally follow current ASTM guidelines (E-1688)

Will be conducted for 28 days with three replicates per test sediment

N. virens (3 to 15 grams wet weight) will be obtained from Aquatic Research Organisms, Hampton, New Hampshire. Will be tested in 10 gallon aquaria with 2 cm of sediment and 8 gallons of overlying water. Salinity will be 10‰ and the tests will be conducted in a temperature controlled room at 20°C. Overlying water will be aerated constantly and changed three times per week. Twenty worms will be tested per replicate. No feeding.

L. plumulosus (2 mg dry weight after 28 days) will be obtained from in-house cultures. Will be tested in 5 gallon aquaria with 2 cm of sediment and a few cm of overlying water. Salinity will be 10‰ while temperature will be 25°C. Overlying water will be aerated constantly and changed three times per week. We are starting some experiments to determine whether *L. plumulosus* can survive 28 days without feeding so we have not made a decision on feeding during the test.

Chronic TIEs

All of the current TIE whole sediment methods are based on short-term acute tests measuring survival as the endpoint.

There is a current method for a 28-d *L. plumulosus* chronic toxicity test using survival, growth and reproduction as endpoints. As you saw earlier, we have used this method extensively in our Baltimore Harbor toxicity assessments.

We have found some sites in Baltimore Harbor which are not acutely toxic but which effect some of these other sublethal endpoints.

Problem: There is no current EPA guidance on these chronic TIE methods.

During the first year of this project we will investigate the feasibility of using these same acute whole sediment manipulations but in a longer term tests, resulting in chronic whole sediment TIE methods.

Chronic TIEs (Cont.)

Kay Ho and her group at EPA's Narragansett, RI laboratory are currently looking into methods for chronic TIEs. Their initial research seems to indicate that the manipulations do not work for the 28 days required for the chronic tests.

We will collaborate with this group in our efforts to design a chronic TIE. Some of the possible avenues for research include:

Can we transfer amphipods to new manipulated sediments at some time during the exposure period without upsetting their growth and reproduction? These are very durable organisms that don't seem to be bothered by physical manipulation.

Can we use a shorter exposure period to yield chronic endpoints? There is currently a freshwater 10-d survival and growth amphipod tests using the amphipod *Hyaella azteca*. Can we use a similar test design with *L. plumulosus* that would give comparable results to the current 28-d test?

If a chronic TIE method can be designed, we will conduct chronic whole sediment TIEs in the second year of the project at 5 sites.